

A STUDY OF CULTURES ISOLATED FROM THE RHIZOSPHERE OF FERULA
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Abstract. *F. kuhistanica* wide is one of the medicinal plants with pharmaceutical potential, and it is possible to further increase these possibilities by studying the microorganism communities in its rhizosphere. This article provides information about the results obtained by isolating microorganisms from the root part of the plant *F. kuhistanica*, studying the structure of colonies, preparing a preparation for microscopy, and studying the morphological characteristics of the culture by staining the cells by Gram method.

Keywords: *Ferula*, rhizosphere, isolation, colony, bacteria, culture, Gram (+), Gram (-), inoculant

Аннотация. *F. kuhistanica* широкий относится к числу лекарственных растений с фармацевтическим потенциалом, и дальнейшее увеличение этих возможностей возможно за счет изучения сообществ микроорганизмов в его ризосфере. В статье представлена информация о результатах, полученных при выделении микроорганизмов из корневой части растения *F. kuhistanica*, изучении структуры колоний, приготовлении препарата для микроскопии, изучении морфологических особенностей культуры путем окрашивания клеток методом Грама.

Ключевые слова: Ферула, ризосфера, изоляция, колония, бактерия, культура, Грам(+), Грам(-), инокулянт.

Explanations and Abbreviations:

Example 1: SN - It was isolated from the experimental area established in the territory of the Sogdiyona neighborhood of Sh.Rashidov district;

Example 2: GSF – It was isolated from the experimental field established in the territory of Gallaorol specialized forestry;

Example 3: LASRI - Rhizosphere microorganisms obtained from the roots of *F. kuhistanica* planted in the experimental field established on the territory of Lalmikor Agricultural Scientific Research Institute

INTRODUCTION

Ferula L. species belonging to the Apiaceae family belong to the group of medicinal plants. Likewise, they are essential oil, fodder, honey-rich, medicinal, starchy, aromatic, nutritious, and technical plants. 200 species are known to science in the world. There are more than 110 species of representatives of this group in Central Asia, about 50 species in Uzbekistan, and about 28 species in the Jizzakh region [11].

Due to the widespread use of species of the *Ferula* genus in folk medicine, their area is shrinking. Currently, glue (resin) is extracted from the roots of *F. foetida* and *F. kuhistanica* plants, and hundreds of tons of raw materials are prepared and exported every year. This situation leads to the depletion of plant resources. Therefore, the introduction of *ferula* species is one of the most urgent problems.

F. kuhistanica is endemic to Central Asia and is included in the pharmacopoeias of the USA, Sweden, Germany, and Portugal. In Uzbekistan, it is used to improve the quality of poultry

eggs, extend the laying period (panaferol), and treat gynecological diseases (tefestrol). [9] It is also used in the treatment of skin diseases and wounds. [3] Due to the high content of ether oils, animals do not eat them well when they are wet. However, it is considered a nutritious feed for livestock in the dry state in the winter. [6]

A large amount of pectin substances, hemicellulose, glucose, mannose, arabinose, rhamnose, and galactose were isolated from the vegetative organs of *F. kuhistanica*. [5] Also, *F. kuhistanica* is rich in ethers, monoterpene hydrocarbons, water-soluble polysaccharides, and pectin substances and is considered an important source for the pharmaceutical industry.

The formation of the root microflora of plants is important for the formation and accumulation of the above-mentioned biologically active substances in the *F. kuhistanica* plant.

In the scientific literature, the rhizosphere bacteria that stimulate the growth and development of plants are called PGPR (plant growth-promoting rhizobacteria), that is, bacteria of the root environment that improve the growth and development of plants.

Common characteristics of plant growth-promoting rhizobacteria (PGPR) are: 1) survival after seed inoculation; 2) reproduction in the spermosphere (the part on the surface of the seed) as a response to root exudates; 3) firmly settling along the root surface and colonizing the growing root system. PGPR colonizes the rhizosphere of a wide range of agricultural crops and participates through direct or indirect mechanisms in stimulating plant growth and development, reducing the number of phytopathogens, and increasing resistance to stress factors (biocontrol). Direct mechanisms of plant stimulation (acceleration of growth and development) include the dissolution of difficult-to-assimilate nutrients in the soil and the synthesis of phytohormones, volatile compounds, vitamins, and amino acids that enhance plant development. [7]

2. Materials and methods

2.1. Collection of plant samples

The plants were dug up with a titanium spade. Shaken. It was placed in polythene bags along with the soil remaining on the roots. A label is affixed with the type of plant, date, time, and location of sampling.

2.2. Insulation

A standard protocol (E.V. Dubrovskoy et al. 2013) was used for the isolation of rhizobacteria. In the laboratory, the roots were separated and thoroughly washed in a stream of faucet water. They were then transferred to a 1 L flask containing 0.5 L of sterile faucet water and washed with shaking on a shaker for 0.5 h. The procedure was repeated three times. The washed roots were transferred to a sterile Petri dish and cut with scissors. They were treated with a physiological solution, and the root rhizosphere was isolated. Cultures were grown in petri dishes with GPA medium for 3-5 days, then transferred to test tubes with nitrogen-free Ashby's medium and incubated at 30 °C for 3-5 days. After that, pure cultures were isolated by Koch method dilution.

2.3. Preparation of slides from isolated samples and Gram staining

A slide was prepared from a culture of microorganisms grown in a Petri dish. The slide was first dried in air, then fixed in a flame and cooled. It was stained with gentian violet and incubated for 2 minutes. The slide was treated with a lugol solution for 2 minutes without washing. After that, the lugol solution was poured, and the slide was washed. It was dried with filter paper. 96% ethanol was dropped for decolorization and washed with a gentle stream of sterile water after 30 seconds. It was incubated with fuchsin dye for 2 minutes. Then it was

washed with water and dried using filter paper. The prepared slide was covered with a cover glass, 1 drop of immersion oil was dropped on it, and it was viewed under a microscope.

Results

Characterization and microscopy of bacterial colony cultures

Three groups of isolated samples formed colonies with similar cultural characteristics on the surface of solid nutrient medium.

Colony signs	Colony number
	1,2,3
Size (mm)	1-1.5
Color	yellow
Shaped	round
Surface	raised
Clarity	half clear
Structure	small grainy
From the side appearance	bulging came out
Consistency	soft
Edge	flat whole

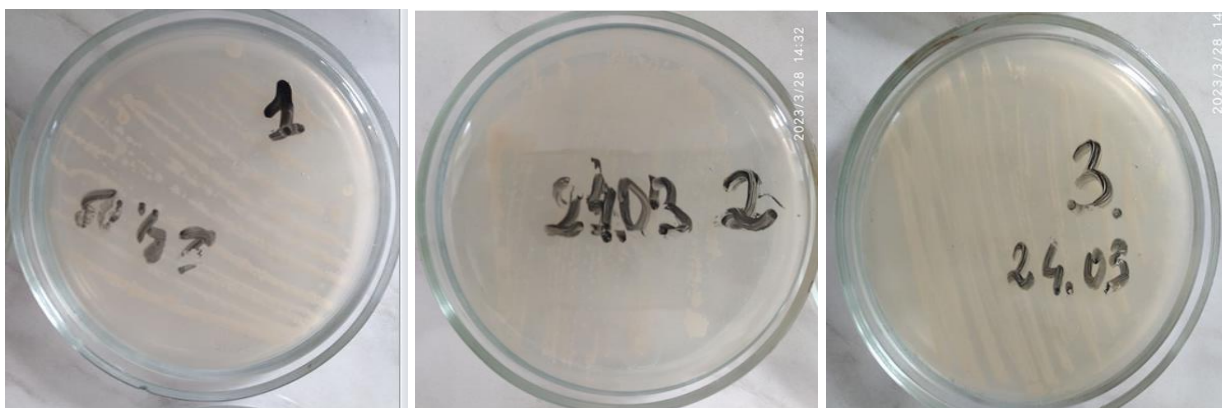


Figure 1. Microbial colonies isolated from the rhizosphere of *F. kuhistanica*

1 from the experimental area SN, 2 from GSF, and 3 from the experimental site LASRI

When the preparations stained by the Gram method were viewed under a microscope (Bio Blue. Lab BB.1153 Pli Euromex), it was found that there were Gram (+) and Gram (-) bacteria of different shapes and sizes in three groups of samples.

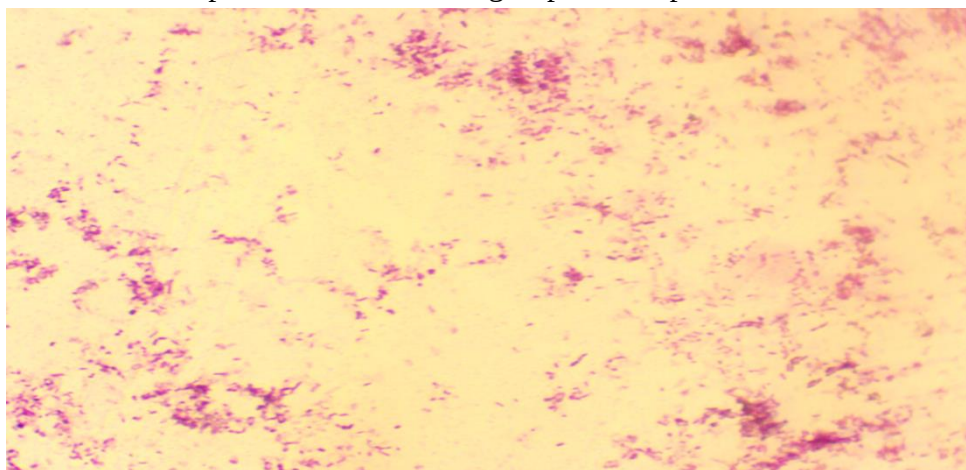


Figure 2. Example 1

As can be seen from figure 2, the bacterial cells in this colony are smaller than the other two samples, oval in shape, and mostly dark in color.

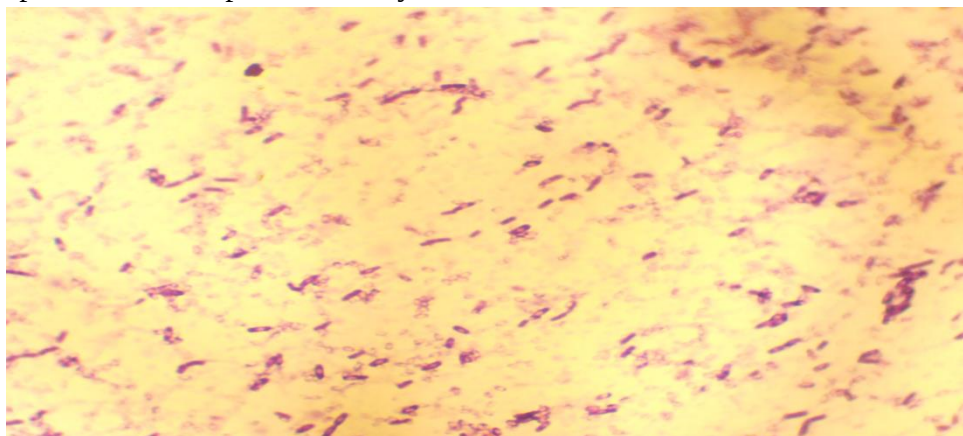


Figure 3. Example 2

According to figure 3, the colony consists of dark-staining large rod-shaped and light-staining small oval-shaped bacterial cells.

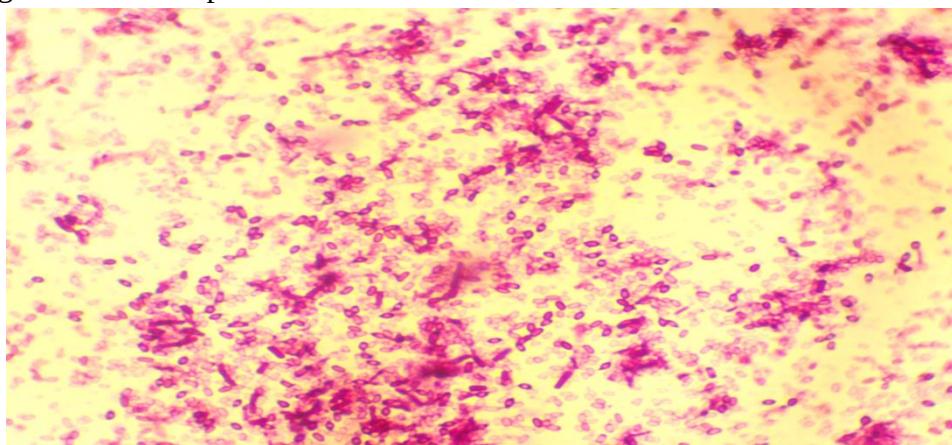


Figure 4. Example 3

From figure 4, it is known that there are Gram (+) and Gram (-) bacteria of oval shape in the culture.

In conclusion, in the formation of important pharmaceutical compounds such as esters, monoterpene hydrocarbons, essential oils (pinene, sabinene, limonene), ferutidine, ferutinin, kufestrol, and kuhistanicaol H, I, and J isolated from different parts of the *F. kuhistanica* plant, the communities of microorganisms in its rhizosphere may have an indirect effect. When the microbial communities of *F. kuhistanica* grown in experimental fields located in different regions were studied, they formed similar colonies, but after staining by the Gram method, it was determined that these colonies contained different sizes and shapes of Gram (+) and Gram (-) bacteria.

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